

Application No. 10/734,609
Amendment dated April 12, 2005
Reply to Office Action of December 1, 2004

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently amended) A method for preparing alphaviral replicon particles (ARPs), said method comprising the steps of:
 - (a) introducing an alphavirus replicon nucleic acid into a host cell, said replicon nucleic acid comprising at least a virus packaging signal and at least one heterologous coding sequence expressible in said alphaviral replicon nucleic acid, wherein said host cell comprises at least one helper function, to produce a modified host cell;
 - (b) culturing said modified host cell under conditions allowing expression of the at least one helper function, allowing replication of said alphaviral replicon nucleic acid and packaging of said alphaviral replicon nucleic acid to form ARPs, wherein said ARPs are heparin -binding ARPs, and
 - (c) contacting the modified host cells after step (b) with an aqueous solution having an ionic strength of from 0.2 M to 5 M to release the ARPs into the aqueous solution to produce a ARP-containing solution;
 - (d) collecting ARPs from the ARP-containing solution of step (c).
2. (Currently amended) The method of claim 1, wherein the at least one helper function in the host cell of step (a) ~~5x10⁷ to 5x10⁸ per mL~~ is encoded by a nucleic acid sequence stably integrated within the genome of said host cell.
3. (Original) The method of claim 1, wherein the at least one helper function in the host cell is introduced on at least one helper nucleic acid which encodes a capsid protein capable of binding said alphaviral replicon nucleic acid, and at least one alphaviral glycoprotein, wherein said alphaviral glycoprotein associates with said alphaviral replicon nucleic acid and said capsid protein, wherein the at least one

Application No. 10/734,609
Amendment dated April 12, 2005
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helper nucleic acid molecule is introduced into the host cell together with said alphaviral replicon nucleic acid.

4. (Original) The method of claim 1, wherein the at least one helper function is encoded by at least two helper nucleic acid molecules wherein each of said two helper nucleic acid molecules encodes at least one viral helper function.
5. (Original) The method of claim 1, wherein the ionic strength is between 0.5 M and 5 M.
6. (Original) The method of claim 1, wherein the at least one helper function is encoded within a DNA molecule.
7. (Original) The method of claim 1, wherein the alphavirus replicon nucleic acid is introduced into said host cell by electroporation.
8. (Currently amended) The method of claim 8, 7, wherein host cells are present in an electroporation mixture at a concentration from 5×10^7 to 5×10^8 per mL.
9. (Currently amended) The method of claim 9, 3, wherein host cells are present in an electroporation mixture at a concentration from 5×10^7 to 1.5×10^8 per mL.
10. (Original) The method of claim 1, further comprising a cell washing step, prior to step (c) of claim 1.
11. (Original) The method of claim 1, wherein the alphavirus is Venezuelan Equine Encephalitis Virus.
12. (Original) The method of claim 10, wherein the cell washing solution contains no salt and further comprises deoxyribonuclease.

Application No. 10/734,609
Amendment dated April 12, 2005
Reply to Office Action of December 1, 2004

13. (Original) The method of claim 3 or 4, wherein the helper nucleic acid is an uncapped RNA molecule.
14. (Currently amended) The method of claim 4, wherein an alphavirus replicon RNA and a first helper RNA molecule and a second RNA helper molecule are present in an electroporation mixture at a ratio of ~~1:0.3:0:0.3 to 1:20:20~~, 1:0.3:0.3 to 1:20:20.
15. (Original) The method of claim 14, wherein the ratio is 1:0.5:0.5.
16. (Currently amended) The method of claim 15, 14, wherein the ratio is 1:5:5.
17. (Original) A method of preparing alphavirus replicon particles comprising introducing an alphavirus replicon vector and one or more helper nucleic acid molecules into alphavirus-permissive cells via electroporation, wherein concentration of the alphavirus permissive cells in culture medium during electroporation is from 5×10^7 to 5×10^8 cells/mL and wherein the concentration of the alphavirus RNA replicon vector added to the cells prior to electroporation is approximately 35 μ g per mL.
18. (Currently amended) The method of claim 11, 17, wherein the electroporation is carried out in an electroporation chamber wherein a gap between electrodes is between 0.4 and 1.0 cm.
19. (Currently amended) The method of claim 11, 17, wherein the helper function is encoded within a single DNA helper molecule encoding all alphavirus structural proteins.

Application No. 10/734,609
Amendment dated April 12, 2005
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20. (Currently amended) The method of claim 45, 19, wherein the concentration of the DNA helper molecule is at least 100 μ g/mL.
21. (Original) The method of claim 1, wherein the alphavirus-permissible cells are Vero cells.
22. (Original) The method of claim 1, wherein step (d) is followed by an ion exchange, heparin affinity chromatography, affinity or hydrophobic chromatography step.
23. (Original) The method of claim 1, wherein the alphavirus is an attenuated alphavirus.
24. (Original) The method of claim 17, wherein the alphavirus is Venezuelan equine encephalitis virus (VEE).
24. 25. (Currently amended) The method of claim 1, wherein the salt in the salt wash step is selected from the group consisting of NaCl, KCl, MgCl₂, CaCl₂, NH₄Cl, (NH₄)₂SO₄, NH₄ acetate and NH₄ bicarbonate.
25. 26. (Currently amended) A method for preparing alphaviral replicon particles (ARPs), said method comprising the steps of:
 - (a) introducing an alphavirus replicon nucleic acid into a host cell, said replicon nucleic acid comprising at least a virus packaging signal and at least one heterologous coding sequence expressible in said alphaviral replicon nucleic acid, wherein said host cell comprises at least one helper function, to produce a modified host cell, wherein said replicon nucleic acid is introduced into the host cell by electroporation of host cells at a concentration of from 5x10⁷ to 5x10⁸ cells per milliliter;

Application No. 10/734,609
Amendment dated April 12, 2005
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- (b) culturing said modified host cell under conditions allowing expression of the at least one helper function, allowing replication of said alphaviral replicon nucleic acid and packaging of said alphaviral replicon nucleic acid to form ARPs;
- (c) contacting the modified host cells after step (b) with an aqueous solution having an ionic strength of from 0.2 M to 5 M to release the ARPs into the aqueous solution to produce a ARP-containing solution;
- (d) collecting ARPs from the ARP-containing solution of step (c).

26. 27. Canceled.

27. 28. (Currently amended) The method of claim 17, 26, wherein the alphavirus is Venezuelan equine encephalitis virus (VEE).

28. 29. (Currently amended) The method of claim 25, 26, wherein the helper nucleic acid is an uncapped RNA molecule.

29. 30. (Currently amended) An alphavirus replicon particle preparation prepared by the method of any of claim 1.